EXHIBIT E

TO DECLARATION OF SCOTT D. TANNER, PHD.

U.S. Patent Application Ser. No. 10/614,115

Peer Review Panel Summary and Recommendation

Strengths

- Very strong technology that could be broadly applicable
- Proof of principle has been achieved
- Strong academic/industrial collaboration
- Applicants responded well to panel concerns

Weaknesses

- Co-funding partner will be crucial to success
- Not convinced that technology will help with leukemia prognosis
- Creation of high-throughput reagents was less convincing

Summary

This proposal represents a very strong and innovative combination of talents and expertise from disparate but highly complementary research groups. The objective of the development of a new, uniquely sensitive and specific method for detection of leukemic stem cells is extremely attractive and will most likely be achieved.

Recommendation

The panel recommends funding the proposal subject to the applicant replacing co-funding that was lost prior to panel review

Budget

- Project funding recommended is a maximum of $8,462,065 over 3 years
- Genome Canada's maximum contribution being $4,231,033 over 3 years

Peer Review Panel Comments

Competition on
Applied Genomics and Proteomics
Research in Human Health

Lead Genome Centre: Ontario Genomics Institute

Principal Investigator: Dick, John
Project Title: Mass Spectrometer- based Flow Cytometer, Methods and Applications

Project Summary: This proposal will develop an advanced flow cytometer based on ICP-MS to better detect the presence of specific cell types in a cell population. The approach is based on the tagging of aptamers or other affinity reagents with specific elements that can be detected at high sensitivity using ICP-MS. This instrument will be used to detect the presence of rare cancer stem cells within populations of leukemia cell populations. Initial applications will focus on the detection of subpopulations of leukemic stem cells since they are the best characterized of the cancer stem cells. The project will deliver a prototype mass spectrometer based flow cytometer, appropriately tagged affinity reagents, methods and demonstrative data for the distinction of various forms of leukemia.

The Research Proposed

The proposed research is taking advantage of existing mass spectrometry technology to create a new approach for the differentiation of cell populations. Inductively couple plasma has been used for quite some time to vaporize, atomize, and ionize elements for detection. ICP has been combined with optical detection methods and the pioneering work of Fassel and Houck the method was coupled to a mass spectrometer to use *m/z* detection of ionized elements. ICP-MS is characterized by high sensitivity and specificity and is used to detect the presence of elements down to the ppb or ppt level. Merging ICP-MS with a flow cytometry based separation method is an outstanding idea and most of the risk in this proposal is in the design and screening of affinity reagents to be used with the detection system. Because the method depends on the use of affinity reagents to introduce the labels, development of highly specific reagents is key. MDS has developed methods to covalently attached elements to proteins and thus this technology exists. MDS-Sciex has a significant track record in the development of mass spectrometers and thus there is likely to be little risk in the

development phase of the instrument. There are some critical choices to be made on the type of mass analyzer to be used. If a quadrupole is used then high selectivity and sensitivity is possible using SRM type measurements and if a TOF is used high resolution measurements are possible allowing resolution elements and isotopes. The inability to select and pass specific *m/z* values directly to the detector may have some issues related to background and dynamic range. A benefit to ICP- TOF is the ability to detect multiple signals simultaneously allowing the analysis of many different proteins in one experiment. The method is limited by the availability of appropriate elements and the available mass space.
Affinity reagents will be developed using an aptamer approach. By evolving RNA in a test tube to bind to specific molecules, highly selective affinity reagents can be developed. Identifying appropriate aptamers is dependent on a good high throughput screening capability and the project has individuals with specific expertise in aptamer and screening technology. Very large libraries can be constructed and then screened against binding targets. A live cell screening procedure is proposed to identify cell surface targets and microarray analysis is proposed to identify binding targets derived from signaling pathways. This task of the project is high risk. Identifying unique cell surface proteins and establishing they are in fact unique will be a major challenge. The live cell screening procedures proposes an approach to uniquely identify binding agents unique to the cell surface of cancer stem cells.
The proposal meets the programmatic needs of the Genome Canada RFA. This is clearly a proteomic approach that will develop new tools for better study and detection of cancer. The ability to detect different subpopulations of cancer stem cells would allow the ability to potentially stratify different types and stages of leukemia and better predict the type of treatment that would be successful.
The research project very clearly draws on the strengths of Canadian science and industry and is a unique contribution. ICP-MS instruments are quite common, but are not generally used in proteomic research. This project also adds the strengths of MDS in the use of element ligands on proteins. MDS is quite knowledgeable in the use of radioisotopes with antibodies and that experience and knowledge will be important.

The Research Team
The research team is excellent. MDSSciex has extensive experience building mass spectrometers and this should be an easy project for them. Yingfu Li is early in his career but with proper guidance from the more senior investigators should do fine. John Dick and Mark Minden are accomplished scientists and capable of conducting the analysis of stems cells proposed in the project.

Environment
The environment at Sciex for instrument development is excellent. The environment at the Ontario Cancer Institute is excellent.

Training
A training plan is briefly outlined discussing the number of post doctoral fellows that will be involved in the project. The training consists primarily of providing seminars. The project will clearly provide some unique training opportunities for students and post docs in the area of proteomics.

Management

A detailed management plan is presented. A scientific advisory group will be formed to direct and manage the science and an International SAB will be formed to evaluate progress on a more formal basis. The plan looks sound. An IP strategy is provided as well as a commercialization plan. Of all the projects this one seemed to be capable of spinning out products in fairly short timeframe. A detailed terms sheet is provided to layout dispute resolution, commercialization terms, and IP management. A data release plan was provided to release non-confidential data via the web.

Relevance
The project has important economic and health benefits for Canada. An instrument and reagents for high sensitivity and selective biological detection would be a nice product for Sciex.

Financial
- **Budget Recommendation:**

Budget proposed is fine. Please review the budget request and make a formal recommendation that is consistent with your scientific recommendations. If you recommend budget cuts, give details and clear reasons for the cuts.
Provide comments on the reasonableness of a project's budgeted costs, the plausibility of the justifications provided for budget items and the effectiveness of financial and budgetary control processes proposed.
Is the request in line with the research proposed and do the benefits of the research justify the budget requested?
- **Co-funding:** MDS-Sciex is funding half of the research.

Rating:

A

Summary Statement:

The most exciting part of the proposal is the use of ICP-MS as a cell type specific detector (or just a specific multiplexed detector). The project describes an innovative use of ICP-MS to create a multiplexed detector that will have a wide variety of uses. MDS's expertise integrating metalligands into proteins is a real plus. The hard part of the grant is the creation of aptamers as validated highly specific affinity ligands. This technology has successfully applied by others but there is some risk that useful affinity reagents will be difficult to identify. The intended biological application of the technology sounds interesting.

Other Issues:
It is a stretch to state that US mass spectrometry companies receive research and development funding from the US government.

External Reviews:

#1
Rating: A
Descriptor: Excellent Summary Statement
This is a very exciting and highly innovative proposal from a superb group of investigators. The objectives of the project directly address important scientific needs, both in the proteomics arena and in medical science. Consequently, the technology holds the potential to strongly impact human health care worldwide. Importantly, the Canadian investigators participating in this project possess a blend of expertise that is entirely unique, and unlikely to be replicated anywhere else. Thus, this consortium of scientists is remarkably well positioned to perform the proposed studies.

Rating: A
Summary Statement:
The proposed research project is extremely exciting, based on a set of research groups with unique and perfectly complementary expertise. The ideas that make the basis for the proposal are clever, well thought out and beautifully integrated. The potential for success is excellent and the impact of successful completion of the proposed research project would be huge. I would give this proposal the strongest possible recommendation for full support.

Competition on
Applied Genomics and Proteomics
Research in Human Health

Lead Genome Centre: Ontario Genomics Institute

Principal Investigator: Dick, John
Project Title: Mass Spectrometer- based Flow Cytometer, Methods and Applications

Summary

This is an exciting proposal from an established group of investigators aiming to develop a novel mass spectrometry-based flow cytometer, and to apply it to the study of leukemia stem cells. The technology, if successfully developed, would be very broadly enabling to the research community, and certainly would facilitate the study of rare cancer stem cells. The project is innovative and likely to have high scientific and potentially high future clinical impact.

Assessment

Programmatic

The proposed work addresses a novel strategy to bring flow cytometry an entirely new level, capable of increasing sensitivity and increasing multiplexing capacity. In that current flow cytometry is used widely in research and clinical settings, an advance such as that proposed here would have potential for very high impact. It has a genomic/proteomic bent of sorts, in that the technology would facilitate the systematic characterization of live cells in the clinical or research setting. One aspect of the proposal is the systematic generation of affinity reagents using DNA aptamer technology, and this aspect is certainly responsive to the genomic focus of this Genome Canada initiative.

Scientific

There are 3 basic components to this proposal: 1) the development and characterization of an ICP- MS flow cytometer, 2) the purification of leukemia

stem cells and genetic characterization therein, and 3) the systematic generation and characterization of affinity reagents using DNA aptamers. The clinical theme of the project is that the ability to characterize cancer stem cell populations in tumors using a highly sensitive measure such as the proposed instrument would have tremendous value as a diagnostic tool useful for guiding patient therapy. While I am enthusiastic or how it is that detection of cancer stem cells would be of obvious clinical importance. This may in fact be correct, but it is certainly not a given.

Regarding the development of the ICP-MS instrument, this reviewer cannot fully judge the technical approach being proposed, but it appears that the effort is being embraced with great seriousness and attention to the possible pitfalls and challenges inherent in the approach. The work is being conducted by seasoned scientists with significant corporate experience, infrastructure and resource behind the project. These, combined with the biological and clinical direction provided by others involved in this proposal, are likely to result in success.

Regarding the stem cell studies, Dr. Dick, who is perhaps the foremost authority on the subject, will isolate leukemic stem cells from animal models, and use those together with expression profiling to characterize these stem cells. This will yield candidate markers to which affinity reagents will be prepared by Dr. Li, which, once developed, would be tested on the new flow cytometer. There is little doubt that Dr. Dick will succeed in the proposed experiments - they are straightforward, and in line with the sorts of experiments occurring routinely in his laboratory.

The affinity reagent development effort is also important and novel, and if successful, could point to a powerful strategy for the development of detection agents. Preliminary data suggesting that the proposed aptamer-based methods really are up to the task of generating affinity reagents on a systematic basis is lacking, but this is balanced by the novelty of the approach, and is probably worth pursuing.

As alluded to above, it is not entirely clear why cancer stem cells were chosen as the training ground for instrument development. There are all sorts of potential uses for the proposed flow cytometer, and working out its performance in a simpler biological system (e.g. cell lines) would seem sensible. Presumably such work will also be performed, even if not explicitly stated in the written application. The applicants would be well-advised to explore the application of a prototype instrument broadly, rather than focusing only on the stem cell application.

Research Team

A real strength of this proposal is the research team, which has world-class expertise in mass spec instrument development and cancer biology. Dr. Li, leading the aptamer portion of the project is a younger investigator, but is nonetheless accomplished, and is deemed to be very well-suited to carry out the proposed experiments. Dr. Minden will guide the clinical studies, and he is well-versed in such translational projects. One could not hope for a better team.

Environment

The environment is outstanding for the proposed work. It contains the desirable elements of industry resource at MDS SCIEX, basic biology laboratories (Dick, Li), and clinical environments (Minden).

Training

The training environment is excellent.

Management

There is a reasonable management plan, including a mechanism for obtaining outside advice. The
written application reflects prior thinking about the multidisciplinary and multi-investigator nature of the proposal.

Relevance
The development of the proposed highly, multiplexable flow cytometer has the potential to have
quite high impact in the research and clinical arena. The relevance is therefore very high. Clearly there is much to learn about cancer stem cells in general, increasingly recognized as being important in understanding tumor biology, bJt the direct impact on human health of the ability to detect cancer stem cells routinely is at this time more speculative.

Financial,

The budget appears appropriate as proposed, although it is not clear that given the numbers of leukemia stem cell purifications to be performed by the Dick laboratory, additional renovation and MoFlo instrument are needed above and beyond those resources already existing in the laboratory for this purpose.

External Reviews

The external reviews are uniformly lauditory.

Rating

A-

Summary Statement

This is an innovative application that nicely merges new technology development, systematic characterization of important cell populations (cancer stem cells), and its exploration in the context of clinical questions. The proposal is well thought out and the team is excellent. It is an excellent application.

Competition on
Applied Genomics and Proteomics
Research in Human Health

Lead Genome Centre: Ontario Genomics Institute

Principal Investigator: Dick, John
Project Title: Mass Spectrometer- based Flow Cytometer / Methods and Applications

PROJECT SUMMARY

This project seeks to achieve the distinction and identification of subpopulations of leukaemic stem cells in patient samples, with the ultimate goal of enabling peronalised therapeutic prognosis. Three areas of science underpin the proposal: recognition of the existence and significance of leukaemic stem cells, prior development of aptamers as tags for the specific identification of target proteins in a quantitative manner, and development of inductively coupled plasma (ICP) mass spectrometry (MS) as an adjunct to aptamer-tagging to achieve unparalleled sensitivy and selectivity of detection. The outcomes of the project will include a new instrument otpimised for selective protein detection, together with a demonstration of the feasibility of using aptamers for selective detection of target proteins. Furthermore, success with all aspects of the project will represent a proof-of-principle concerning the feasibility of detection of cell types that relate directly to prognosis in acute myeloid leukaemia.

ASSESSMENT

The Research Proposed

Programmatic. The proposed programme is large-scale and has the potential to have a very great impact on health care, particularly in the developing field of personalised medicine in the context of leukaemia diagnosis and treatment. The research is appropriately classed as 'post-genome science' by virtue of the fact that suitable protein markers of cell type will be provisionally identified by microarray (i.e. transcriptome) analysis of highly purified cell populations. It is, however, an overstatement to suggest (as the applicants do) that they will be defining a 'proteomic signature' of each cell type; analyses at the protein level will

only validate (albeit as an essential part of the strategy) the candidate markers suggested by microarray analysis. This quibble over terminology (which, I should say, troubles neither of the referees) is important only in that there is a risk of overstating what is proposed and should not detract from the excellence of the planned research.

Scientific.

This proposal represents a highly imaginative development upon the very substantial recent research achievements of the participating laboratories. Dr Dick has been responsible for important conceptual developments in the cell biology of leukaemia, which have suggested the need for, and potential benefits from, highly sensitive and specific cell counting techniques. Dr Li's expertise and contributions in the aptamer field suggest new ways forward in developing highly specific cell labelling methods. Finally, the expertise in ICP-MS in Dr Tanner's laboratory at MDS-Sciex, together with his recent developments in its use for biopolymer analysis, open the way to increased specificity and sensitivity of detection of tagged cells. Thus, one of the most appealing aspects of the current proposal is the combination of expertise from previously disparate fields, namely the application of ICP-MS, a tool previously used primarily for inorganic elemental analysis, to research in cell and molecular biology. In a sense, this conceptual leap has already been made by the MDS Sciex members of the present group of applicants, who recently described quantitative element-tagged immunoassay with ICP-MS detection. The present proposal is a novel extension of this approach, incorporating very important additional ideas.

The project is divided into a number of "activities", which map rather precisely onto the participating laboratories. Dr Dick and his immediate colleagues will perform purifications of the cell types of interest. RNA amplification and global gene expression analysis will allow the identification of candidate proteins to differentiate haemopoietic stem cells and leukaemic stem cells. Functional assays will be employed to assess the significance of the candidates. The work required to meet these objectives is very extensive - and full development of other aspects of the programme are dependent upon their success. Nevertheless, the feasibility within the stated timescale is reasonable.

An initial feasibility study will be performed using isolated cell populations where the cells will be permeabilised and incubated with commercial antibodies (tagged with gold) to known cell-surface and internal protein markers. Analyses at this stage will be performed using an ICP-quadrupole MS. High sensitivities will be achieved by single ion monitoring (it being recognised that this instrument type is largely satisfactory for sensitive targetted analysis of a single element). Observation or otherwise of the internal tagged protein will provide an indication

of the success of complete ionisation of the cell preparation. Whereas the arguments in favour of subsequent use of aptamers rather than antibodies to provide specificity of tagging are well developed, the consideration of the effectiveness of aptamer labelling of internal proteins in the cell populations to be characterised is not considered in detail..

The number of aptamers to be developed as a consequence of the screening (at transcriptome level) of potential targets is not specified; it is an omission that prevents full consideration of the scale of the work involved.

Activities 4 and 5 relate to the selection, optimisation and validation of aptamers. This work includes application of a new approach to in vitro aptamer selection that benefits from solid-phase coupling to permit the selection of aptamers based on binding affinity. A complementary approach will employ in vivo aptamer selection using live cells (where the identified targets are premably exclusively cell surface-bound). Referee 1 notes the difficulty of the work with whole cells, judging from previous experience with antibody selection. Finally, aptamers will, where possible, be raised against purified proteins. While these experiments in molecular biology are outside my immediate expertise, the
proposals appear both innovative and feasible. Certainly, the focus on the use of aptamers rather than antibodies seems well justified (though the proposal recognises that some preliminary experiments using antibody labelling may be appropriate).

The development of the mass-spectrometry-base flow cytometer is described in detail, with the discussion indicating a clear understanding of the major issues and potential pitfalls. The preference for incorporation of time-of-flight separation of ions (in an orthogonal configuration) is well considered. Highly multiplexed detection of multiple tagged proteins, a key feature of the proposed approach, would not otherwise be possible. The possibility has been recognised that the residence time in the ICP source may be insufficient to achieve complete ionisation (to atomic levels) of the introduced cells. This may be particularly significant if the detection of tagged intra-cellular components is required (though note the comments made above). Accordingly, the possible need for on-line cell lysis has been considered and a couple of sensible options are proposed. Should this development be a necessity, however, it is likely to represent an area requiring considerable development. The applicants have also considered the need to alleviate the effects of high charge density immediately following the ICP source; modified ion optics are proposed.

The Research Team

The research team is first-rate. Dr Dick has a very strong record of recent achievement in the elucidation of leukaemia cell biology. Dr Minden will

contribute particularly with respect to clinical aspects. Dr Li has established his academic career in Canada relatively recently but has some exceptional achievements to his name in the area of aptomer research. Finally, Dr Tanner brings exceptional skills and experience to the development of ICP-MS and the novel application proposed here; as noted above, the previous work of Dr Tanner and his colleagues achieved the essential intellectual leap, corresponding to the application of ICP-MS to biopolymer detection, that underpins much of the current proposal.

Environment

The close proximity of each of the participating laboratories will facilitate productive interaction. Each component of the proposal is a logical extension of individual research programmes in the applicants' laboratories so there is no doubt concerning the commitment to the programme. In particular, it is very clear that the industrial partner, MDS-Sciex, is totally committed, as evidenced by the recent resolution at Board level.

Training

A total of 3 postdoctoral fellows, 3 technicians and 4 research associates are proposed to be added to the academic component of the programme, representing a significant opportunity for enhanced training of new individuals in these research areas. Seminars involving all the partner laboratories will be held on an annual basis (arguably of insufficient frequency) and these will contribute to both internal training and broader outreach. MDS-Sciex have a strong record of fundamental in-house research so that this component also represents an important training opportunity for the scientists involved.

Management

The proposed management structure is robust. Appointment of a programme manager is proposed and this is well considered. A Management Committee will be responsible for items not directly related to the science. Much of the latter will be determined by individual project leaders. A process has been established to resolve differences of opinion between project leaders. There will be a Science Advisory Group, which will assess scientific priorities; this group (the deliberations of which should surely mini mise the risk of differences of opinion over scientific priorities) is intended to meet "at least annually". Annual meetings would be insufficient and commitment to more frequent meetings should be made. External guidance will be provided by an Scientific Advisory Council, for which annual meetings *are* appropriate.

Relevance

Two clear categories of benefit accrue from the proposed research. In the first place, the development of new diagnostic todls for leukaemia treatment will have very great healthcare benefits, both within Canada and beyond. Second, the co-funding of research establishing a new area of application of ICP-MS will benefit MDS-Sciex as an important component of Canadian industry.

Financial

Just over 50% of the projected budget is to be met by MDS-Sciex through coverage of internal personnel and research costs. The detailed real personnel costs of the proposed work appear to have been considered in great detail and the budget appears fully justified. The evident commitment of MDS-Sciex, the sole co-funders, ensures the stability of co-funding.

RATING A, Excellent, in agreement with both referees, who express very high levels of enthusiasm for this proposal.

SUMMARY STATEMENT

This proposal represents a very strong and innovative combination of talents and expertise from disparate but highly complementary research groups. The objective of the development of a new and uniquely sensitive and specific method for detection of leukaemic stem cells is extremely attractive and will plausibly be achieved. Whereas the suggestion that the work adopts a "proteomic" approach to this problem is questionable, the research is undoubtedly embedded in post-genome science.

OTHER ISSUES

None.

Competition on
Applied Genomics and Proteomics
Research in Human Health

Lead Genome Centre: Ontario Genomics Institute

Principal Investigator: Dick, John
Project Title: Mass Spectrometer- based Flow Cytometer, Methods and Applications

The application presented by Dick et al. proposes to develop an advanced flow cytometer instrument with an inductively coupled plasma mass spectrometer detector to simultaneously analyze cell surface properties, intra cellular signaling and transcriptional pathways on single cells. Considering that cancer in general and leukemia in particular is a stem cell disease where cancer stem cell sustains the tumor and drives its growth, immortality and malignancy, the applicants want to separate an characterize leukemia stem cells and downstream progenitors. They will be used for detection of highly specific non cross reactive aptamers. Tagging these aptamers with distinct stable isotope tags is also part of their project. The group will develop a purpose-specific system combining single cell sample introduction and mass spectrometry instrument based on the time of flight configuration.

Their goal is to ultimately develop a prototype mass spectrometer-based flow cytometer and appropriately tagged affinity reagents for distinction and treatment of various forms of leukemia.

This research project is extremely exciting and convincing, owing to complementary expertise of the various research groups associated to this application. The chances of success are solid, the impact for public health is certain and the benefits for Canadian economy, industry and health care are obvious.

Rating: A.

Competition on
Applied Genomics and Proteomics
Research in Human Health

Lead Genome Centre: Ontario Genomics Institute

Principal Investigator: Dick, John
Project Title: Mass Spectrometer-based Flow Cytometer, Methods and Applications

1. Are there ethical, environmental, economic, legal or social issues (GE3LS) associated with this project? What are these?

This project appears to concentrate on the development of an innovative and original instrument to improve research. If successful, this new instrument will have a significant impact on research and the Canadian Biotechnology industry. Most of the work is laboratory based and does not appear to raise substantial ethical issues.

The documentation for the project only refers to patient samples on one occasion (at page 17). One of the team will lead the studies on patient samples from the 00 cell bank. There will have to be a check on these samples to ensure that the samples are covered by appropriate consent and the samples de-identified. If the samples are not de-identified privacy issues are involved.

The project does not involve susceptibility testing. There is no indication that personal, clinically relevant information will be uncovered with this research.

2. Have the project leaders identified all the relevant GE3LS?

The project leaders have not specifically addressed GE3LS issues. The researchers say they will delegate a representative from the project to participate in the OGI GE3LS program. They also state that they will "identify/review and discuss any GE3LS issues"

3. Is there a plan to address these issues? Is the plan adequate? Are there deficiencies? Is there appropriate expertise including the group to address these issues?

There is a brief section in the application (P26) on these issues.

4. If GE3LS issues have not been addressed, please indicate the actions you consider necessary to address them.

4.1 Individual consent and involvement of biological relatives and families

The samples provided by OCI should be covered by appropriate consent for use. This will have to be checked.

4.2 Privacy, access by third parties including coding in databanks

The issue of identification of the samples requires clarification. The 00 will almost certainly have de-identification procedures that protect privacy enabling the samples to be provided in de-identified form.

If there are any privacy issues, these must be addressed and any proposal approved by the relevant REB.

4.3 Disclosure of potential harms to the RES and how these harms are addressed as part of the research project.

There does not appear to be any potential harms to any individuals arising from this laboratory based research.

4.4 Access to genetic counseling where appropriate.

It is presumed that the original sample providers will already have received disease specific counseling.

4.5 Banking of genetic material and issues of confidentiality, privacy, storage, use of the data and the results, withdrawal by the subject, and future contact of subjects, families and groups.

If the data or material will be banked, issues of confidentiality, access to information, storage, release will arise. However, it appears that the identification information will be held by 00 and not the

researchers.

4.6 Disclosure to RES of potential commercial uses of genetic material. Required.

4.7 Other matters

Applied Genomics and Proteomics Research in Human Health Competition

External Review of Large Scale Project

Lead Genome Centre: Ontario Genomics Institute

Project Leader(s): Dick, John - University Health Network,

Project Title: Mass Spectrometer-based Flow Cytometer, Methods and Applications

Programmatic

Studies described by Dick and colleagues propose to develop a novel instrument and technology for proteomic analysis of cancer stem cells. The proposal is very timely in that the importance of cancer stem cells has recently received significant attention in the medical and scientific literature. It is clear that prognostic/diagnostic tools that specific focus on cancer stem cells would be of enormous value to the oncology community. Thus, the technology described has the potential to strongly impact human health care in Canada and the rest of the world.

Scientific

By any measure this is a remarkable proposal. The proposed studies will develop a novel and highly sophisticated technology to address an essential issue in cancer biology. Notable strengths are a very creative and exciting technology platform, a unique blend of scientific expertise, and the potential to revolutionize the way in which cells are analyzed. Moreover, if successful in the context of the present application (i.e. for cancer stem cells), the potential for widespread application in other areas is very strong.

The research plan consists of three project areas: 1) identification and characterization of normal vs. leukemia stem cells, 2) development of novel aptamer-based affinity reagents, and 3) development of a mass spectrometer system to detect elemental signatures on individual cells. The first project will be led by Dr. John Dick and will consist of a detailed analysis of normal vs. leukemia stem cells. Using state of the art technologies, Dr. Dick will generate highly specific immunophenotypic profiles for stem cell populations and employ in vivo animal models as a means to validate such profiles. Based on his previous work, all the proposed studies are entirely feasible and likely to generate a wealth of

important new data. Most importantly, the immunophenotypes will provide the antigenic "picture" on which the aptamer and mass spectrometer technologies will ultimately focus. The second project will be led by Dr. Li and will generate DNA aptamers for specific protein targets. Studies will employ both whole cells as well as purified proteins as the targets for aptamer affinity selection procedures. The use of whole cells is likely to be problematic, given the difficulties encountered for similar studies attempting to isolate phage antibodies. However, use of purified proteins is well validated and likely to succeed. Similarly, development of appropriate chemistry for coupling of DNA to specific elements appears to be feasible. Thus, the general objectives of project #2 are feasible and likely to provide important reagents for the overall project. Finally, the third project will develop and validate the MS-flow cytometer. While this reviewer is not qualified to assess the technical aspects of project #3, based on existing technology and the parameters described, the overall efforts appears highly worthwhile.

The Research Team

An important strength of this application is the superb research team. Dr. Dick is an internationally respected leader in the stem cell biology field and has pioneered the analysis of human and leukemic stem cells. He is quite simply the most qualified person in the world to conduct the proposed studies. Moreover, the support of Dr. Minden, also an outstanding investigator, lends further strength to the biological aspects of the project. Dr. Li is a younger investigator but has distinguished himself as an outstanding scientist and appears extremely well qualified to conduct the DNA aptamer studies. Finally, the corporate interaction with MDS Sciex and Dr. Tanner also appears to be excellent.

Environment

The environment for each of the projects is outstanding. Moreover, the proximity of investigators in Ontario lends itself to facilitating strong interdisciplinary interactions.

Management

The overall management structure appears logical and sufficient for a project of the scope proposed. Plans for a dedicated project manager are regarded as very important and will likely be critical to success of the project. The oversight committees will also lend useful support to the effort. Notably, the establishment of a separate science advisory group (SAG) and an international science advisory council (I SAC) will provide a broad base on valuable guidance and feedback. Although the two bodies may be somewhat redundant, given the breadth of the proposed studies, it will probably be important to receive as much input as possible.

Rating: A

Descriptor: Excellent

Summary Statement

This is a very exciting and highly innovative proposal from a superb group of investigators. The objectives of the project directly address important scientific needs, both in the proteomics arena and in medical science. Consequently, the technology holds the potential to strongly impact human health care worldwide. Importantly, the Canadian investigators participating in this project possess a blend of expertise that is entirely unique, and unlikely to be replicated anywhere else. Thus, this consortium of scientists is remarkably well positioned to perform the proposed studies.

Applied Genomics and Proteomics Research in Human Health Competition
External Review of Large Scale Project

Lead Genome Centre: Ontario Genomics Institute

Project Leader(s): Dick, John - University Health Network

Project Title: Mass Spectrometer-based Flow Cytometer, Methods and Applications

Project Summary:
The proposal is to develop a new instrument that will provide massively-multiplexed assays for protein signatures that will allow multiplexed disease state classification for cancer from measurements of cells. The proposed instrument is a combination of two powerful techniques, flow cytometry and quantitative, highly selective elemental chemical analysis by inductively coupled plasma mass spectrometry. The new instrument, along with newly developed tagged (aptamer-based) affinity reagents will be applied to distinguish various forms of leukemia as a demonstration of the potential of the technique to detect and characterize rare cancer stem cells.

Assessment:
The Research Proposed Programmatic. The proposed research has a dear proteomics focus including seeking a unique proteomic signature of individual leukemic stem cells and then using that information to determine how normal human hematopoietic stem cells are converted into leukemic stem cells. The new "tool" will is likely to improve the prediction, prevention and treatment of human disease. It would also seem that this approach will be generally applicable to other types of cancer as well. The proposed research certainly holds great promise to enable personalized therapeutic treatment for different subclassifications of leukemic patients. The proposed research is certainly large-scale and has great potential to directly impact human health in a timely manner.

Scientific. The questions to be addressed are important and clearly formulated. The proposed instrument is very likely to enable new observations and knowledge related to disease state classification for cancer from individual cells which in turn could lead to new approaches to personalized therapy. The new instrument and chemical tags could also be fruitful in obtaining a better understanding of cancer development particularly, but not exclusively, in cases where rare stem cells are key to sustaining the cancer. The project appears to be well coordinated, integrated and inclusive with a set of unique expertise and

laboratories working on different portions of the project with clear contributions needed from each. The proposed experiments including instrument design, building and testing, affinity tag development and application to leukemic samples are well thought out to address the key questions. The methods and data analyses appear to be appropriate. The main challenges for the instrument development include efficient coupling of the flow cytometer and ICP-MS, development of the time-of-flight MS specific for this purpose and complete vaporization of fairly large cells during the 2-5 ms they spend in the ICP. The proposal includes sufficient consideration of alternative approaches, including the possible need of on-line cell lysing ,to convince the reviewer that development of the new instrument will very likely be successful. The milestones are well thought out and feasible. The goals are likely achievable. The proposal is very innovative and original. The beauty of the newly proposed instrument is that it is a combination of two techniques that previously were used by very different disciplines but which together provide exciting new capabilities. Flow cell cytometry is widely used in biological applications while ICP-MS is typically used for inorganic elemental analysis. However, ICP-MS provides high *selectivity,* high sensitivity, a huge linear dynamic range and accurate, precise quantification that is not obtainable by more commonly used detectors for flow cell cytometry. This new tool could enable entirely new applications to proteomics, fundamental cancer research and disease treatment thereby having a significant impact on Canada's capacity for innovation as well as providing an opportunity for this group of Canadian researchers to become world leaders. If successful, Canada will provide the new capabilities to the rest of the world through manufacturing of the new instrument "tool" including selective element tags. The proposal is a uniquely Canadian contribution that capitalizes on existing Canadian strengths as well as likely enhancing an area where Canada is currently a not leader (aptamer based medical diagnostic technology) but has an active group of researchers that are poised to become leaders. MDS Sciex is a Canadian company that is a world leading manufacturer of ICP-MS instruments. It has lead the way in bioanalytical analysis using element tags and ICP-MS detection. It is also a leader in mass spectrometers for organic and biological mass spectrometry including time-of-flight instruments. The group also includes leaders in cancer stem cell investigation and chemical recognition of biological markers.

The Research Team

The research team is world-class in each of the key areas of the proposal. No where else in the world is there such a perfectly complementary group of world leaders. Dr. Scott Tanner is well known as one of the leading ICP-MS experts in the world. His unique, extensive expertise in mass spectrometry and ion-molecule reactions has lead to unique, world-leading ICP-MS instruments from Sciex. During the last two years Scott, together with an impressive group of co-workers at Sciex, has lead the ICP-MS community and Perkin Elmer Sciex in an entirely new direction with the application of specific tags and elemental mass

spectrometry to solve bioanalytical problems. The support for instrument development at Sciex is also an advantage of the team to be working on the proposed research. Dr. Ralph Sturgeon, who expressed *interest* in collaborating with this group, also brings unique expertise and experience. He has reported impressive results with an ICP- TOF-MS for elemental analysis so he will *likely* contribute useful insight to some of the practical, technical issues. Dr. John Dick has a long list of superb accomplishments in the area of cancer research, most recently focused on rare leukemic stem cells and their role in leukemia. Dr. Li in is unique in leading a Canadian research group in the area of aptamer research technology. The expertise of this group is essential to the success of the proposal and its world wide reputation will be further enhanced by their involvement in the proposed research. Dr. Minton's group at OCI provides excellent experience for successful testing of the new instrument and tagging system in a clinical setting. All of the participants in the proposal have been very productive recently and in the past. It is difficult to imagine a better more complementary group of researchers for the proposed research.

Environment

The scientific environment at each of the collaborators' institutions is superb for the proposed research. The proposed experiments certainly take advantage of the unique features of each group; each group's contribution is both unique and essential to the proposed research. Furthermore, there is enough overlap of interests among the groups so that the unique contributions of each can be transfered, shared and integrated to successfully pursue and attain the goals of the proposal. There is clear evidence of support from each of the participating organizations for the proposed research project.

Training

Clearly, the University based institutions provide an environment that encourages training of graduate students, postdoctoral researchers and medical researchers. Sciex is well known as a company that thinks from a fundamental, academic-like scientific basis and has a prior history of educating through publications of fundamental science, involvement in scientific conferences, support of university research and interactions with leading academic researchers and their students. Hopefully, this approach, which is in contrast to many instrument manufacturers, will continue or even grow with collaborations like those in this proposal.

Management

The proposed management plan is appropriate and effective. Each of the collaborating groups has experience in effectively managing large research projects. The detailed management plans certainly anticipate how decisions will be made, priorities set, division of responsibilities, etc. The plan for resources and deployment of human resources, equipment, etc., from the initial ramp-up through completion of the proposed project are well thought out. Detailed plans

for data release and dissemination of results and data are well conceived and appear to strike an appropriate balance between issues of academic researchers need to publish, including student theses with the need for confidential information that is essential to commercial development. The plan for technology transfer and commercialization is ideal with in the involvement of Sciex and PerkinElmer Sciex, who have a clear motivation for development to a commercially viable product.

Relevance

There are clear economic, industrial and health benefits to Canadians. The successful research promises to have a major enabling impact on fundamental understanding of human disease, particularly cancer and personalized therapy. A successful instrument and specific elemental tags along with clear demonstration of the applications of the technique would undoubtedly lead to a commercial instrument system that would be a successful Sciex product.

Financial

The proposed research requires development of start of the art instrumentation, new highly selective affinity tags and sophisticated biochemical experiments and evaluation on clinical samples. The budget proposed to carry out the series of experiments, developments and tests is adequate and appropriate. Certainly the financial support commited by PerkinElmer/MDS Sciex joint venture demonstrates the seriousness of their interest in enhancing and ensuring the success of the proposed research at an appropriate level.

Rating: A

Summary Statement:

The proposed research project is extremely exciting, based on a set of research groups with unique and perfectly complementary expertise. The ideas that make the basis for the proposal are clever, well thought out and beautifully integrated. The potential for success is excellent and the impact of successful completion of the proposed research project would be huge. I would give this proposal the strongest possible recommendation for full support.